THE EFFECT OF POTASSIUM, NITROGEN AND PHOSPHORUS FERTILIZING UPON THE CHLOROPLAST PIGMENTS, UPON THE MINERAL CONTENT OF THE LEAVES, AND UPON PRODUCTION IN CROP PLANTS[1]

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Introduction

In a former paper (**8**) by the writer, the effect of nitrogen on the chloroplast pigments has been especially indicated. The effect of nitrogen upon the growth of plants is perhaps greater than that of any of the other elements.

Ville (**14**) was perhaps the first to make any quantitative experiments on the effect of the various fertilizers upon the pigment content of plants. He has shown that nitrogen plays a rôle more important than that of any of the other elements tested. If the dose of nitrogen was increased or diminished the color increased or diminished also.

Urban (**13**) found evidence of a correlation between the color of the leaves of beets and the nitrogen content, the darker leaves containing the most nitrogen and the lighter the least. Also, he apparently found a relation between the highest potassium content of the leaves and the greatest sugar content of the roots. In beets the dark colored leaves contained more potassium and less sodium than did the light colored leaves. In the leaves of ripening beets the potassium rapidly increases while the sodium decreases.

A great difference in the chlorophyll content of alpine and of lowland plants was observed by Henrici (**2**). She represented the amount of chlorophyll present in alpine plants as 100 per cent. and found that lowland plants contained 230 per cent. and ravine plants 350 per cent. She makes no reference to the soil in which these grew but it is highly probable that the soil was a very important factor in the amount of chlorophyll which was found in the plants studied.

Perhaps the first real experiments on the effect of chemical fertilizers upon chlorophyll were conducted by Wlodek (**16**) in 1920. Green leaves of potato plants and sugar beets were studied. These were grown in soils to which various fertilizers were added: (1) without fertilizer; (2) with phosphorus, potash and nitrogen; (3) with phosphorus and nitrogen but no potash; (4) with phosphorus, nitrogen and magnesium but no potash; (5) with potash and nitrogen but no phosphorus; and (6) with potash and phosphorus but no nitrogen. After a certain period of development of the

[1] Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

plants it was concluded that the relation of the chlorophyll pigments varies during the course of 24 hours; β increases during the day and α during the night. The lack of potash resulted in an absolute and relative diminution of β and an increase in α as well as a reduction in the daily variation of the two components. Lack of phosphorus also reduces the daily variation of the chlorophyll components and narrows the absorption bands. Nitrogen tends to reduce α and to augment β. The action of calcium and magnesium were not definitely established.

In another paper WLODEK (**17**) has reported that with a lack of potash the chlorophyll coefficient diminished at a certain phase of development of the plants. This is shown by an increase in the width of the absorption band of chlorophyll α and a decrease in that of chlorophyll β, as compared with plants grown under normal conditions. A lack of nitrogen in the soil increases the chlorophyll coefficient. A relation seems to exist between the width of the first absorption band and the nitrogen content in fresh bean leaves, and in the straw and harvest of oats and barley. The nitrogen content increases with the width of the absorption band. When potash is lacking the chlorophyll coefficient does not change under the influence of light and darkness. In leaves which show an abnormal chlorophyll coefficient the amount of vegetative material which is produced is less than in those which possess the normal chlorophyll coefficient.

In studying the bush characteristics of potato plants, SCHAEFER (**7**) has placed the leaves in four groups as to color. The classes were, bright green, green, green to dark green, and dark green. He thought that possibly nitrogen affected the color.

WEISSMANN (**15**) noticed that potash influenced the development of plants and their morphological and anatomical structures. He found also that deficient potash affects the coloring of the leaf, stem and the grain. Deficient phosphorus and nitrogen more strikingly affect the early stages of growth than does a deficiency in potash.

In making chemical analyses of leaves, CHIBNALL (**1**) learned that there was a nitrogen withdrawal from the leaves at night. This fact may aid us in understanding the nitrogen metabolism of the leaf.

Studies by MAIWALD (**3**) lead to the conclusion that different applications of potassium produced a great difference in the color of the potato leaves of the same species. The leaves varied in color from a dark green to a yellow green, the yellow green ones resulting when much potash was applied. When based on green weight, deviations of the chlorophyll content were found to be 70 per cent. above and below normal. The amount of the chlorophyll present was the sole cause of the striking color difference in the leaves. By comparing the assimilating power of the plant with the chlorophyll content, the author concludes that the abnormally low chlorophyll content is the beginning of chlorosis.

Potassium sulphate was found to reduce the chlorophyll content of sound green potato leaves, while kainit and potassium chloride were found by REMY and LIESEGANG (**5**) to greatly reduce the amount of chlorophyll present. Healthy leaves of pronounced potash hungry plants always contained more chlorophyll per unit weight than potash satisfied plants; the same is also true of sugar beets. Leaves of potatoes which had been treated with large amounts of potash salts contained less chlorophyll than did leaves from potatoes receiving less potash salts. Plants treated with potash have a larger growth than plants not treated, consequently in spite of the fact that the content of chlorophyll per gram of leaf is less, each plant actually has more chlorophyll in it. Leaves of plants fertilized with potash possess the power to assimilate for a greater length of time than do those not fertilized.

In studying the effect of nitrate applications upon the hydrocyanic acid content of sorghum, PINCKNEY (**4**), found that nitrogen affected the size and the color of the plants to such an extent that sorghum may be used as an indicator of the amount of available nitrogen in the soils. Plants grown in soils containing readily available nitrogen grew rapidly and were much greener in color than plants grown in soils which did not contain available nitrogen.

Sir JOHN RUSSELL (**6**) has discussed the effects of nitrogen, phosphorus and potash upon plant growth. Nitrogenous fertilizers increase the rate of leaf growth and so produce larger leaves and stems. Also, they induce a greater formation of green coloring matter, giving darker green crops. Phosphates on the other hand greatly increase the root growth.

Methods and materials

The methods used for extracting and separating the pigments were those described in a previous paper (**11**) by the writer. The amount of chlorophyll present was measured by the colorimetric method outlined in the paper on the quantitative determination of chlorophyll (**12**). The carotinoids were estimated by means of the colorimetric methods (**9, 10**), devised by the writer. The spectrophotometric methods were not used here for they had not been developed at the time the work described in this paper had been completed.

The materials used were collected from various field fertilizer experiments of this department. The cotton leaves were from the field experiments of J. J. SKINNER and the potato leaves from those of B. E. BROWN. An attempt was always made to collect representative samples of each fertilizer plot. The samples were forwarded in waxed paper, to prevent drying of the sample, to Washington, D. C., and the analysis was completed in this laboratory.

The numbers 1, 16, 21, 7, 8, 9, and 10 in the tables refer to that portion of the P. N. K. fertilizer triangle, in use in this department, from which the samples were taken. The composition of these fertilizers is shown in the following table I.

TABLE I

Composition of fertilizers used in these studies

Treatment number	Formula		
	NH_3	P_2O_5	K_2O
1	0	20	0
16	0	0	20
21	20	0	0
7	0	8	12
8	4	8	8
9	8	8	4
10	12	8	0

The elements N, P, K, and S, were determined by the following methods, which need not be described in detail here. Total nitrogen was determined by the Kjeldahl method as modified by Gunning and Arnold while total phosphorus was estimated by the Neumann-Pemberton method. Sulphur was estimated by the fusion method, and potash was estimated by the Assoc. of Offic. Agr. Chemists' method substantially as given in the year 1919.

Results

The pigment results were all obtained by a direct comparison with Lovibond slides, the readings from which were later interpreted in terms of grams of pigment. Part of the leaves were dried and part of them were extracted in the fresh condition. For the chemical analysis the leaves were all dried according to regular laboratory procedure. The dried leaves for extraction were then ground to a fine powder with sand in a ball mill before extraction.

In general, a survey of tables II and III shows that the plots fertilized with a mixture high in phosphorus produced leaves which contained less chlorophyll than did leaves from plots high in potash or nitrogen. Leaves from plots high in nitrogen contained more of the chloroplast pigments than did the leaves from plots from other parts of the triangle.

Plants fertilized with a mixture high in nitrogen always produced plants whose leaves were also high in nitrogen. The plants which were fertilized

TABLE II

ANALYSIS OF DRIED COTTON LEAVES[1] COLLECTED AT NEWBERN, NORTH CAROLINA, JULY 23, 1919

NUMBER OF PLOT	1	16	21	7	8	9	10
Chlorophyll ($\alpha+\beta$)	21.0	26.8	29.4	28.6	22.4	31.8	31.8
N	4.97	5.22	6.03	5.14	5.09	5.31	5.50
P	0.72	0.52	0.50	0.60	0.61	0.49	0.50
K	1.34	1.88	2.09	1.37	1.86	1.99	2.33
S	1.35	1.32	1.18	1.43	1.31	1.44	1.23
Pounds of cotton per acre	1424.0	1080.0	2176.0	1434.0	1802.0	2262.0	2528.0

COLLECTED AT FLORENCE, SOUTH CAROLINA, JULY 26, 1919

Chlorophyll ($\alpha+\beta$)	21.0	25.0	26.0	17.6	27.6	25.0	25.0
N	2.98	3.30	5.25	3.13	3.48	4.60	4.91
P	1.03	0.52	0.32	0.37	0.34	0.48	0.51
K	0.89	1.79	1.15	1.68	1.54	1.25	1.17
S	1.52	0.96	1.05	1.49	1.53	1.17	1.05
Pounds of cotton per acre	644.0	770.0	1662.0	888.0	1426.0	1948.0	1850.0

PEE DEE EXPERIMENTAL STATION

COLLECTED AT FLORENCE, SOUTH CAROLINA, AUGUST 29, 1919

Chlorophyll ($\alpha+\beta$)	22.4	23.2	31.8	20.2	18.4	29.4	31.0
N	2.71	3.12	4.62	2.50	2.46	2.31	3.82
P	0.61	0.48	0.36	0.50	0.37	0.31	0.32
K	1.70	2.70	1.14	1.86	1.44	0.98	1.03
S	1.18	1.09	0.74	0.95	0.64	0.62	0.84

COLLECTED SEPTEMBER 21, 1919

Chlorophyll ($\alpha+\beta$)	24.2	15.8	22.0	16.6	23.2	17.6	26.8
Pounds of cotton per acre	1780.0	1640.0	2280.0	1372.0	1676.0	2150.0	2130.0

[1] The results reported for the chloroplast pigments are given in milligrams per 10 grams of fresh leaves. The chemical analyses are reported in grams of N, P, K or S per 100 grams of dry leaves, *i.e.*, in per cent. Data obtained for the carotinoids are not given, for drying the leaves renders these data worthless.

with a mixture high in phosphorus always produced plants whose leaves were high in phosphorus. Plants which were fertilized with a mixture high in potash always produced cotton plants with leaves high in potash. Generally the leaves of plants high in nitrogen contained the least sulphur while those high in phosphorus contained the most sulphur.

TABLE III

ANALYSIS OF FRESH COTTON LEAVES, PEE DEE EXPERIMENT STATION. COLLECTED AT FLORENCE, SOUTH CAROLINA, JUNE 25, 1920

NUMBER OF PLOT	1	16	21	7	8	9	10
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Carotin	0.66	0.90	0.86	0.57	0.63	0.76	0.76
Xanthophyll	1.20	1.70	2.00	2.00	1.50	1.50	1.30
Total carotinoids	1.86	2.60	2.86	2.57	2.13	2.26	2.06
Chlorophyll ($\alpha+\beta$)	19.0	26.8	27.4	22.0	23.4	23.0	23.4
COLLECTED SEPTEMBER 29, 1920							
Carotin	0.70	0.78	0.90	0.78	0.86	0.86	0.75
Xanthophyll	1.60	1.03	1.16	1.00	1.30	1.50	1.16
Total carotinoids	2.30	1.81	2.06	1.78	2.16	2.36	1.91
Chlorophyll ($\alpha+\beta$)	16.6	14.8	16.6	13.6	14.8	14.8	16.00
Pounds of cotton per acre	1520.0	1040.0	938.0	1600.0	1608.0	2004.0	1620.0

In general, it may be concluded that plots high in potash gave low yields of cotton while those high in nitrogen produced the most cotton per acre, although this is not true for all types of soil. The phosphorus plots produced yields which were between those for potash and nitrogen.

A summary of the results from the potato plots (table IV) shows that the plots fertilized with a mixture high in phosphorus produced leaves which contained less chlorophyll than did leaves from plots high in nitrogen. Leaves from plots high in potash evidently contained less chloroplast pigments than did leaves from the other plots.

Potato plants fertilized with a mixture high in nitrogen produced plants whose leaves were high in nitrogen. Plants which were fertilized with a mixture high in potash produced plants whose leaves contained the least potash. Plants fertilized with a mixture high in phosphorus apparently produced leaves which were also high in phosphorus. The amount of sulphur in the leaves could not be correlated with the amount of nitrogen, phosphorus, or potash present in the leaves or that added to the soil as fertilizer.

In table V results are given for a fertilizer experiment at Arlington Farm. In this case, the results are not at all in conformity with what might have been expected and the chloroplast pigments cannot be correlated in any way with the amount of fertilizer added. However, the table shows that as the plants matured the total carotinoids increased from 2.5 to 3.27 mg. as also did the total chlorophyll from 12.8 to 15.3 mg. in each

TABLE IV

ANALYSIS OF POTATO LEAVES, DRIED BEFORE EXTRACTION

IRISH COBBLER VARIETY

COLLECTED MAY 29, 1919, NORFOLK, VIRGINIA

NUMBER OF PLOT	1	16	21	7	8	9	10
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Chlorophyll (α + β)	15.5	15.9	18.0	....	....	....	....
COLLECTED JUNE 17, 1919, NORFOLK, VIRGINIA							
Chlorophyll (α + β)	16.8	15.2	21.1	18.5	16.4	15.9	19.4
Yield (bushels per acre)	81.0	73.0	97.0	75.0	178.0	193.0	167.0
GREEN MOUNTAIN VARIETY COLLECTED JULY 15, 1919, RIVERHEAD, LONG ISLAND							
Chlorophyll (α + β)	26.0	19.2	32.8	21.0	28.6	35.2	31.8
Yield (bushels per acre)	176.0	191.0	91.0	166.0	192.0	181.0	187.0
NORCROSS VARIETY COLLECTED AUGUST 4, 1919, PRESQUE ISLE, MAINE							
Chlorophyll (α + β)	12.6	32.8	35.2	39.6	35.2	34.6	38.6
N	4.87	4.60	6.02	4.69	5.15	5.44	6.23
P	0.35	0.27	0.36	0.32	0.21	0.31	0.39
K	2.63	2.08	2.37	2.06	2.09	1.81	....
S	0.35	0.34	0.36	0.33	0.33	0.41	0.39
No yields available							
IRISH COBBLER VARIETY COLLECTED AUGUST 4, 1919, PRESQUE ISLE, MAINE							
Chlorophyll (α + β)	19.2	14.0	18.4	14.0	26.0	14.8	20.2
N	4.49	5.15	6.00	4.71	5.10	5.91	5.41
P	0.37	0.30	0.26	0.29	0.29	0.39	0.34
K	2.34	2.25	2.53	3.83	3.75	3.10	1.45
S	0.55	0.72	0.63	0.45	0.71	0.40	0.42
Yield (bushels per acre)	165.0	192.0	153.0	219.0	328.0	336.0	288.0

ten gram sample of the fresh leaves. The samples of leaves which were dried show that the amount of carotin and xanthophyll lost in drying was quite large and consequently the results are unreliable, while the loss of

TABLE V

Chloroplast analysis of fresh potato leaves which were collected at different stages of growth Grown at Arlington Farm, Virginia

Plot number	Date of collection	1	16	21	7	8	9	10	Check	Check dried
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Carotin	August 9, 1920	0.83	0.83	0.82	0.61	0.66	0.80	0.86	0.27	0.41
	August 24, "	0.66	0.73	0.70	0.70	0.70	0.76	0.76	0.72	....
	September 7, "	0.70	0.63	0.15	0.66	0.93	0.74	0.68	0.88	0.56
	September 20, "	0.70	0.70	0.90	0.83	0.70	0.73	0.80	0.93	....
Xanthophyll	August 9, 1920	1.60	2.00	1.60	2.20	2.00	2.00	1.80	1.90	1.00
	August 24, "	1.60	1.70	1.60	1.70	1.60	1.10	1.50	1.20	....
	September 7, "	2.10	2.70	3.50	1.40	1.90	1.80	2.10	2.40	1.30
	September 20, "	1.80	2.60	2.00	2.50	2.50	3.00	2.50	3.00	....
Total carotinoids	August 9, 1920	2.43	2.83	2.42	2.81	2.66	2.80	2.66	2.17	1.41
	August 24, "	2.26	2.43	2.30	2.40	2.30	1.86	2.26	1.92	....
	September 7, "	2.80	3.33	3.65	2.06	2.83	2.54	2.78	3.28	1.86
	September 20, "	2.50	3.30	2.90	3.33	3.20	3.73	3.30	3.93	....
Chlorophyll $(\alpha+\beta)$	August 9, 1920	13.8	12.8	11.8	12.8	12.6	14.8	12.6	11.6	7.9
	August 24, "	11.3	15.6	13.1	11.8	9.5	20.1	12.6	10.9	....
	September 7, "	14.4	14.8	12.7	11.0	15.2	12.7	14.5	16.2	13.6
	September 20, "	15.4	15.4	15.4	15.2	15.4	16.6	15.4	14.1	....

the chlorophyll was much less. This fact shows that the leaves should never be dried if accurate quantitative data are desired for the chloroplast pigments. This table shows also that certain fertilizers do not always produce certain effects upon the development of the chloroplast pigments. The effect then apparently is due to some factor which the fertilizer influences indirectly. Here a potash fertilizer produced plants high in chlorophyll while nitrogen produced plants relatively low in chlorophyll.

The experiments with potatoes, reported in this paper, show that high yields of tubers are correlated with heavy potash fertilization, which also is correlated with a low chlorophyll content of the leaves. Low yields of tubers are correlated with high nitrogen fertilization and a high chlorophyll content of the leaves. High phosphate produces yields of potatoes which are less than those from the high potash plots and greater than those from the high nitrogen plots.

Conclusions

From the above experiments it is clear that only fresh leaves should be used in the determination of the four chloroplast pigments. A very large percentage of carotin and of xanthophyll is lost on drying. The process of drying the leaves is far more destructive to the carotinoids than it is to the chlorophylls.

Phosphorus, potash and nitrogen each may be correlated with an effect on the formation of the chloroplast pigments. Nitrogen was found to be correlated with an increase in the amount of chloroplast pigments which were present in each ten gram sample of fresh green leaves. The nitrogen was also correlated with an increase in the amount of the carotinoids. In potatoes, high potash evidently suppressed chloroplast pigment formation while this was also true in some of the plots of cotton. High phosphorus plots would then evidently produce more chloroplast pigments than potash and less than nitrogen.

Either cotton or potato plants fertilized with a mixture high in nitrogen always produces leaves with a high nitrogen content. Potato plants fertilized with a mixture high in potash produced leaves which contained the least potash, while cotton plants produced leaves which contained the most potash. Both cotton and potato plants which were fertilized with mixtures high in phosphorus also produced leaves which were high in phosphorus. In the case of potatoes the amount of sulphur in the leaves could not be correlated with the amount of nitrogen, potash or phosphorus while in the case of cotton the leaves high in nitrogen contained the least sulphur, and the leaves high in phosphorus contained the most sulphur.

Plots which were high in potash gave low yields of cotton while those high in nitrogen produced the most cotton per acre although this is not true for all types of soil. High yields of potatoes are correlated with heavy potash fertilization which also is correlated with a low chlorophyll content of the leaves. Low yields are correlated with high nitrogen fertilization and a high chlorophyll content of the leaves.

When the results from a fertilizer experiment at Arlington Farm are correlated with results elsewhere it is seen that P, N, and K do not always have the same effect on the chloroplast pigments. At Arlington Farm the potash did not seem to suppress the chlorophyll formation nor did nitrogen increase chlorophyll formation, which is not in agreement with findings elsewhere.

The results given in this paper are in harmony with those of MAIWALD for potatoes. He found that potato leaves from plots fertilized by the addition of potash contained considerable less chlorophyll than leaves of potato plots to which no potash was added. The amount of the chlorophyll rapidly decreased in the potash plots from June to August. In the plots without potash the decrease in the chlorophyll was not so rapid. Leaves from plots fertilized with both potash and nitrogen showed a decided increase in their chlorophyll content. Plants in these plots did not show a decrease in chlorophyll from June to August as did the potash plots.

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